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Association between Cystic Fibrosis Transmembrane Conductance Regulator Gene Mutations and Susceptibility for Childhood Asthma in Korea

Kyung Won Kim,[1] Ji Hyun Lee,[2] Min Goo Lee,[2] Kyung Hwan Kim,[2]
Myung Hyun Sohn,[1] and Kyu-Earn Kim[1]

[1]Department of Pediatrics and Institute of Allergy, Brain Korea 21 Project for Medical Science, Severance Biomedical Science Institute, Yonsei University College of Medicine, Seoul;
[2]Department of Pharmacology, Brain Korea 21 Project for Medical Science, Pharmacogenomic Research Center for Drug Transporters, Yonsei University College of Medicine, Seoul, Korea.

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Corresponding author: Dr. Myung Hyun Sohn, Department of Pediatrics and Institute of Allergy, Brain Korea 21 Project for Medical Science, Severance Biomedical Science Institute, Yonsei University College of Medicine, 250 Seongsan-ro, Seodaemun-gu, Seoul 120-752, Korea.
Tel: 82-2-2228-2050, Fax: 82-2-393-9118
E-mail: mhsohn@yuhs.ac

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Purpose: Classic cystic fibrosis is now known part of cystic fibrosis transmembrane conductance regulator (CFTR)-related disorders. These include a wide spectrum, from multi-system disorders, such as cystic fibrosis, to mono-symptomatic conditions, such as chronic pancreatitis or congenital bilateral absence of the vas deferens. However, respiratory disease is considered typical for the multi system disorder, cystic fibrosis, and is the major cause of morbidity and mortality. The purpose of this study was to evaluate the potential effects of CFTR gene mutations in Korean children with asthma. **Materials and Methods:** We selected 14 mutations identified in Korea and each of the 48 children with and without asthma were genotyped for the case-control study. **Results:** No significant differences were found in genotype and allele frequencies of the 9 polymorphisms observed between the non-asthma and asthma groups. In a haplotype determination based on a Bayesian algorithm, 8 haplotypes were assembled in the 98 individuals tested. However, we also did not find any significant differences in haplotype frequencies between the non-asthma and asthma groups. **Conclusion:** We have concluded that this study did not show any evidence in support of providing that CFTR genetic variations significantly contribute to the susceptibility of asthma in Korean children.

Key Words: Cystic fibrosis transmembrane conductance regulator, asthma, children

INTRODUCTION

Asthma is a chronic inflammatory disorder of the airways characterized by variable airflow obstruction and bronchial hyperresponsiveness.[1,2] The pathogenesis and etiology of asthma are very complex and not fully understood, although an interaction of multiple genetic loci and a variety of environmental factors have been suggested as important determinants.[3-6] Among them, the promising candidate gene is the cystic fibrosis transmembrane conductance regulator (CFTR) gene that is located on chromosome 7q31.2 (http://www.ncbi.nlm.nih.gov/sites/entrez?db=gene).

Mutations in the CFTR gene result in abnormal epithelial ion and water transport and may subsequently incur disturbances in airway mucociliary

clearance.[7] There are more than 1600 CFTR sequence variations registered in the CF mutation database (http://www.genet.sickkids.on.ca/cftr). However, the majority of mutations have been identified in Caucasians, and furthermore, the spectrum of mutations and genetic polymorphism has not been well described in Asian populations. In Korea, the presentation of classical classic cystic fibrosis (CF) is extremely rare and there are a number of reports regarding this subject.[8-10] A few study show that some polymorphisms and mutations of the CFTR gene are associated with respiratory and pancreatic diseases in the Korean population.[11,12]

The purpose of this study was to evaluate the possible effect of the CFTR gene on susceptibility to asthma in Korean children.

MATERIALS AND METHODS

Subjects

48 subjects with and without asthma were recruited from Severance Hospital at Yonsei University for this study, comprised of fifty-seven boys and thirty-nine girls.

Asthma diagnosis was made in accordance with the American Thoracic Society (ATS). In short, current asthma was defined as recurrent wheezing or coughing in the absence of a cold in the preceding 12 months with a physician's diagnosis, and bronchial hyperresponsiveness upon methacholine challenge ($PC_{20} \leq 16$ mg/mL) or at least 12% reversibility of forced expiratory volume in 1 s (FEV_1) after inhalation of β2 agonist.[13,14] Atopy was defined as a positive skin test to more than one extract of the common local aeroallergens, and non-atopy was defined as a negative skin test and serum IgE concentration less than 100 IU/mL. All subjects were enrolled before the administration of oral or inhaled corticosteroids. Patients treated with systemic corticosteroids due to asthma exacerbation in the preceding 6 months were excluded from this study.

Non-asthma subjects were age-matched to healthy children who visited the hospital for general health workups who had no history of wheezing, recurrent or chronic diseases, infection during the preceding 2 weeks, or hypersensitivity to methacholine. Non-asthma subjects also had negative results on the skin prick test for allergens and did not take any medications.[14] All subjects did not have any other disease history including pancreatic diseases. Written consent was obtained from all participants before enrollment in the study, which had been previously approved by the Severance Hospital Institutional Review Board.

Genotyping

Whole blood was obtained from each subject and genomic DNA was extracted by using the QIAmp DNA blood Mini kit (QIAGEN, Hilden, Germany) as decribed.[15] The genotyping was analyzed by a single base primer extension assay using a SNaPShot assay kit according to the manufacturer's protocols (ABI, Foster City, CA, USA), and polymorphisms in the IVS8 TG_n and T_n microsatellites were analyzed by bi-directional nucleotide sequencing. Briefly, the genomic DNA region containing both of the single nucleotide polymorphism (SNP) was amplified with PCR reaction. Each PCR reaction contained: 10.0 ng of DNA, 1X PCR Buffer, 0.125 units of AmpliTaq Gold DNA polymerase (ABI), 3.0 mM $MgCl_2$, 0.25 mM of each dNTP, and 0.5 pmole of each primer in 10 μL reaction volume. Reactions were incubated at 95˚C for 10 min, then cycled 30 times at (95˚C for 30 s, 60˚C for 1 min, 72˚C, for 1 min) followed by 72˚C for 5 min.

After amplification, the PCR products were treated with 1 unit each of shrimp alkaline phosphatase (SAP) (Roche) and exonuclease I (USB Corporation) at 37˚C for 60 min and 72˚C for 15 min to purify the amplified products. One microliter of the purified amplification products was added to a SNaPshot Multiplex Ready reaction mixture containing 0.15 pmoles of genotyping primer. The primer extension reaction was carried out for 25 cycles of 96˚C for 10 s, 50˚C for 5 s, and 60˚C for 30 s. The reaction products were treated with 1 unit of SAP at 37˚C for 1 h and 72˚C for 15 min to remove excess fluorescent dye terminators. One microliter of the final reaction samples containing the extension products was added to 9 microliters of Hi-Di formamide (ABI). The mixture was incubated at 95˚C for 5 min, followed by 5 min on ice and then analyzed by electrophoresis in ABI Prism 3730 DNA analyzer. Results were analyzed using Gene Mapper software (ABI).

Statistical analysis

Statistical analyses were performed using SPSS 11.5 (SPSS Inc., Chicago, IL, USA). Genotype frequency comparisons between asthma and non-asthma groups were performed by chi-square test. Fisher's exact test was used if expected cell frequencies were lower than 5. Genotype frequencies at each SNP were tested for Hardy-Weinberg equilibrium. Haplotypes were assembled by using the software based on the Bayesian algorithm (Haplotyper2). All p values were based on two-sided comparisons and p values of less than 0.05 were considered to indicate statistical significance.

RESULTS

Subjects

The clinical characteristics of the 48 asthma and 48 non-asthma subjects are presented in Table 1. There were no

statistical differences in demographic data such as age and sex between the two groups. However, subjects with asthma were significantly associated with lower lung function ($p < 0.05$). In addition, there were significant differences in total eosinophil counts, total IgE, and serum eosinophil cationic protein (ECP) with atopy-related parameters between the asthma and non-asthma group ($p < 0.01$).

Genotype frequencies in asthma and non-asthma groups

To investigate the association between CFTR genetic variations and asthma, a case-control study was performed using samples from 98 subjects as detailed in Materials and Methods. We genotyped the 14 mutations identified in Korea as summarized in Table 2.[9-11] Diallelic loci were analyzed by automated DNA screening (SNaPshot; Applied Biosystems Inc.), and the TG_n, T_n numbers were identified by bi-directional nucleotide sequencing. Among the 14 mutations, there are no mutant variants in Q98R, I125T, A309, Q220X, and Q1291X loci in our sample and the genotype frequencies of the remaining variants are listed in Table 3. There were no significant differences in genotype

Table 1. Clinical Characteristics of the Study Subjects

Characteristics	Asthma (n = 48)	Non-asthma (n = 48)	*p* value*
Age (yrs; mean ± SD)	9.48 ± 2.04	9.63 ± 2.44	0.753
Sex [Male; n (%)]	33 (71.7)	24 (50.0)	0.037
Lung Function			
FVC (% predicted; mean ± SD)	83.50 ± 10.11	89.18 ± 9.88	0.016
FEV_1 (% predicted; mean ± SD)	77.47 ± 18.98	86.54 ± 10.54	0.010
FEV_1_FVC (% predicted; mean ± SD)	97.91 ± 8.26	105.74 ± 5.87	< 0.001
FEF25-75 (% predicted; mean ± SD)	70.34 ± 20.89	89.45 ± 24.76	0.001
PEF (% predicted; mean ± SD)	78.92 ± 21.49	91.56 ± 27.93	0.028
Methacholine PC20 [mg/mL; n (%)] < 16	48 (100)	0 (0)	< 0.001
≥ 16	0 (0)	48 (100)	
Total serum IgE levels (ln IU/mL; mean ± SD)	5.63 ± 1.84	3.26 ± 1.23	< 0.001
Total Eosinophil count (ln μL-1; mean ± SD)	6.27 ± 0.67	4.92 ± 0.91	< 0.001
Eosinophil cation protein (ln μg/L; mean ± SD)	3.73 ± 1.75	2.44 ± 1.22	< 0.001

FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; FEF, forced expiratory flow; FEF25-75, forced expiratory flow between 25% and 75% of the FVC; PEF, peak expiratory flow.
*χ^2 test or t-test were used where appropriate.

Table 2. CFTR Genetic Variations Analyzed in This Study

Name	Nucleotide change	Exon	Consequence	Reference
- 8G / C	G to C at 125	5' UTR	sequence variation	9
Q98R	A to G at 425	Exon 4	Gln to Arg at 98	8
I125T	T to C at 506	Exon 4	Ile to Thr at 125	9
E217G	A to G at 782	Exon 6a	Glu to Gly at 217	9
Q220X	C to T at 790	Exon 6a	Gln to Stop at 220	7, 8
A309A	C or G at 1059	Exon 7	Sequence variation	9
TG repeat	TG_{10-13}	IVS 8	Splicing	9
T repeat	T_{5-9}	IVS 8	Splicing	9
M470V	A or G at 1540	Exon 10	Met to Val at 470	9
I556V	A to G at 1798	Exon 11	Ile to Val at 556	9
T854T	T to G at 2694	Exon 14a	Sequence variation	9
Q1291X	C to T at 4003	Exon 20	Gln to Stop at 1291	9
Q1352H	G to C at 4188	Exon 22	Gln to His at 1352	9
R1453W	C to T at 4489	Exon 24	Arg to Trp at 1453	9

CFTR, cystic fibrosis transmembrane conductance regulator.
Mutation names and nucleotide numbers are presented according to the Cystic Fibrosis Genetic Analysis Consortium (CFGAC) (http://www.genet.sickkids.on.ca/).

and allele frequencies of the 9 polymorphisms observed between the non-asthma and asthma groups.

Haplotype patterns and their disease associations

Since multiple alleles were analyzed in our study, a haplotype-based approach was applied to find the disease-associated CFTR variations. The Haplotype program based on the Bayesian algorithm was used and Haplotypes were assembled using the genotype data obtained from the 98 tested samples.[16] Nine loci consisting of 7 diallelic variants and two microsatellites of IVS8 TG_n and T_n were analyzed. Since the program accepts only diallelic data, IVS8 TG_n, TG_{10}, and TG_{11} were considered as wild-type (WT), and TG_{12} or TG_{13} were regarded as mutant. For IVS8 T_n, T_5 was considered mutant and other alleles were applied as WT.

After 100 rounds of interactions, 8 haplotypes were assembled and their identification (ID) numbers were assigned according to the total sample frequencies (Table 4). Major haplotypes showing over 1% frequency in both groups are presented in this table. Differences between non-asthma and asthma groups were analyzed by the chi-square analysis. However, no significant differences were found in haplotype frequencies between the two groups.

DISCUSSION

This is the first study to investigate the association between CFTR mutations and asthma in Korean children, and no significant association was found in our pilot study. However, the association between CFTR mutations and asthma is controversial. Mennie, et al.[17] did not find any association between the CFTR gene mutations and asthma in a British population. The lack of significant association between CF heterozygosity and asthma found in the present study is also supported by studies from the French,[18] Italian,[19] Singaporean Chinese,[20] and Norwegian[7] populations. Furthermore, Hakonarson, et al.[21] demonstrated that

Table 3. Frequency of CFTR Genetic Variations in Non-Asthma and Asthma Group

Variants		Non-asthma (n)	Asthma (n)	*p* value*
- 8G / C	G / G	39	37	0.466
	G / C	8	11	
	C / C	1	0	
E217G	A / A	48	46	0.247
	A / G	0	2	
M470V	A / A	8	10	0.858
	A / G	25	23	
	G / G	15	15	
I556V	A / A	42	45	0.276
	A / G	4	3	
T854T	T / T	15	16	0.639
	T / G	26	22	
	G / G	7	10	
Q1352H	G / G	46	46	0.383
	G / C	2	2	
R1453W	C / C	47	46	0.500
	C / T	0	1	
Microsatellite				
TG repeat (IVS 8)†	W / W†	10	12	0.119
	W / M	27	18	
	M / M	10	18	
T repeat (IVS 8)	5 / 7	2	1	0.141
	6 / 7	0	1	
	7 / 7	44	42	
	7 / 9	1	4	

CFTR, cystic fibrosis transmembrane conductance regulator.
*p values were obtained by using the χ^2 test or Fisher's exact test (expected cell value < 5) and the Q98R, I125T, A309, Q220X, and Q1291X variants were excluded from the table because of no frequency.
†TG10 and TG11 were regarded as wild-type (W) and TG12 and TG13 were regarded as mutant-type (M).

Table 4. Frequency of CFTR Haplotypes in Non-Asthma and Asthma Group

Allele ID	- 8G / C	E217G	TG10-13	T_5-7,9	M470V	I556V	2694T/G	Q1352H	R1453W	Group	
										Non-asthma n (%)	Asthma n (%)
1	G	E	WT*	WT†	V	I	T	Q	R	37 (38.5)	30 (31.3)
2	G	E	MT	WT	M	I	G	Q	R	30 (31.3)	28 (29.2)
3	G	E	MT	WT	V	I	T	Q	R	9 (9.4)	15 (15.6)
4	C	E	MT	WT	M	I	G	Q	R	9 (9.4)	10 (10.4)
5	G	E	MT	WT	V	V	T	Q	R	4 (4.2)	3 (3.1)
6	G	E	WT	WT	V	I	T	H	R	2 (2.1)	2 (2.1)
7	G	E	WT	5	V	I	T	Q	R	2 (2.1)	1 (1.0)
8	G	G	MT	WT	M	I	G	Q	R	0 (0.0)	2 (2.1)
									Total	96	96

CFTR, cystic fibrosis transmembrane conductance regulator.
Haplotypes were assembled using software based on the Bayesian algorithm (Haplotyper 2). Major haplotypes showing over 1% frequency in the non-asthma and asthma groups are presented in this table. Haplotype identification (ID) number swere assigned according to the frequency of haploid genes analyzed in this study. Differences between non-asthma and asthma groups were analyzed by the Chi-square analysis.
*TG10 and TG11 were regarded as wild-type and TG12 and TG13 were regarded as mutant-type.
†T6, T7, and T9 were regarded as wild-type.

a study from Iceland failed to show evidence of a linkage between asthma and chromosome 7q31.2.

In contrast, Dahl, et al.[22] found that ΔF508 heterozygosity was associated with an increased susceptibility to asthma in a Danish population. Additionally, studies from Greek[23,24] and Spanish[25] populations reported a positive association between asthma and CF heterozygosity.[24] Schroeder, et al.[26] suggested that obligate ΔF508 carriers are protected from asthma. However the background haplotype for ΔF508,[27] which accounts for 66% of worldwide cystic fibrosis, is very rare in the Korean population.[11]

Besides, genetic variants at Q1352H or E217G were found to be associated with bronchiectasis and/or chronic pancreatitis in the Korean population.[11] In particular, non-synonymous Q1352H and E217G mutations in the M470 background caused a 60-80% reduction in CFTR-dependent Cl^- currents and $HCO3^-$ transport activities. However, we could not find any significant association at those sites in this study. In addition, Q220X and Q1291X mutations that give rise to premature stop codon can lead to aberrant function. However, there are no mutant variants in those loci in our study sample.

Several reports suggested that ΔF508 carriers have lower values of pulmonary function such as FEV_1 or FVC compared to non-carriers, although no difference in the annual decline in lung function was observed between the two groups.[24,28] However, Byard and Davis[29] showed that there are no significant differences in spirometric values between CFTR gene mutation carriers and non-carriers. In this study, we did not have any significant correlation between spirometric values and CFTR gene mutations in the 14 mutations (Table 2, data not shown).

It is worth considering some limitations of our study. The sample size was too small and we did not investigate the full sequence of the CFTR gene. Further study is recommended to verify the results based on our pilot study.

We conclude that this study has failed to produce evidence in support of the notion that CFTR genetic variations identified in the Korean population significantly influences the expression of the asthmatic phenotype.

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